09/712002

* May contain prev. viewed citations

FILE 'CAPLUS' ENTERED AT 14:30:21 ON 06 APR 2001

L1 7 SEA FILE=CAPLUS ABB=ON PLU=ON (MUTANT OR MUTAGEN? OR MUTAT? OR POLYMORPH? OR POLY(W)(MORPHISM OR MORPHIC?))(S) (ENDOSTATIN OR ENDO STATIN)

L1 ANSWER 1 OF 7 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:379117 CAPLUS
DOCUMENT NUMBER: 133:114738
TITLE: Endostatin-induced tyrosine kinase signaling through the shb adaptor protein regulates endothelial cell apoptosis
AUTHOR(S): Dixelius, Johan; Larsson, Helena; Sasaki, Takako; Holmqvist, Kristina; Lu, Lingge; Engstrom, Ake; Timpl, Rupert; Welsh, Michael; Claesson-Welsh, Lena
CORPORATE SOURCE: Department of Genetics and Pathology, Rudbeck Laboratory, Uppsala, S-751 85, Swed.
SOURCE: Blood (2000), 95(11), 3403-3411
CODEN: BLOOAW; ISSN: 0006-4971
PUBLISHER: American Society of Hematology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Endostatin, which corresponds to the C-terminal fragment of collagen XVIII, is a potent inhibitor of angiogenesis. Fibroblast growth factor-2 (FGF-2)-induced angiogenesis in the chicken chorioallantoic membrane was inhibited by **endostatin**, but not by an **endostatin mutant** R158/270A, lacking heparin-binding ability. Endostatin was internalized by endothelial cells, but not by mouse fibroblasts. Treatment of murine brain endothelial (IBE) cells with endostatin reduced the proportion of cells in S phase, whereas growth-arrested IBE cells in collagen gels treated with endostatin displayed enhanced tubular morphogenesis. IBE cells overexpressing Shb, an adaptor protein implicated in angiostatin-induced apoptosis, displayed elevated apoptosis and decreased tubular morphogenesis in collagen gels in response to endostatin when added together with FGF-2. Induction of apoptosis was dependent on the heparin-binding ability of endostatin and the expression of Shb with a functional Src homol. 2 (SH2)-domain. Endostatin treatment for 10 min or 24 h induced tyrosine phosphorylation of Shb and formation of multiprotein complexes. An Shb SH2 domain fusion protein pptd. a 125-kd phosphotyrosyl protein in endostatin-treated cells. The 125-kd component either contained intrinsic tyrosine kinase activity or occurred in complex with a tyrosine kinase. In conclusion, our data show that endostatin induces tyrosine kinase activity and enhanced apoptosis in FGF-treated endothelial cells.

REFERENCE COUNT: 38
REFERENCE(S): (1) Bergers, G; Science 1999, V284, P808 CAPLUS
(2) Boehm, T; Biochem Biophys Res Commun 1998,

Searcher : Shears 308-4994

V252, P190 CAPLUS
(3) Boehm, T; Nature 1997, V390, P404 CAPLUS
(4) Cao, Y; Prog Mol Subcell Biol 1998, V20, P161 CAPLUS
(5) Chang, Z; Am J Pathol 1999, V155, P71 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L1 ANSWER 2 OF 7 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:327473 CAPLUS

TITLE: Using bovine pancreatic trypsin inhibitor as a fusion partner to increase heterologous secretion of endostatin in the yeast S. cerevisiae.

AUTHOR(S): Burbank, Jason A.; Wittrup, K. Dane

CORPORATE SOURCE: Department of Chemical Engineering, University of Illinois, Urbana, IL, 61801, USA

SOURCE: Book of Abstracts, 219th ACS National Meeting, San Francisco, CA, March 26-30, 2000 (2000), BIOT-248. American Chemical Society: Washington, D. C.
CODEN: 69CLAC

DOCUMENT TYPE: Conference; Meeting Abstract

LANGUAGE: English

AB The budding yeast Saccharomyces cerevisiae combines the high cell culture d. and ease of manipulation of bacteria with much of the eucaryotic post-translational processing and secretion machinery found in mammalian cells. Because of this, S. cerevisiae is an ideal system for the prodn. of therapeutic proteins that do not require complex glycosylation. Recently, it has been shown that fusing an aggregation-prone protein to a highly sol. "carrier" protein can increase the soly. of the passenger protein, sometimes dramatically. Our lab has extensively investigated the mechanics of protein secretion from S. cerevisiae using Bovine Pancreatic Trypsin Inhibitor (BPTI) as a model protein. BPTI is a small (58 amino acids), disulfide bond-contg. (3 disulfide bonds) protein that folds compactly and is highly sol. Our lab also recently developed a system for display and directed evolution of combinatorial polypeptide libraries on the surface of yeast, allowing mutants with improved binding to be sorted via flow cytometry. We will construct fusions of BPTI with endostatin and and assay the resulting secretion efficency. We will then seek to improve this secretion efficiency by subjecting BPTI to directed evolution via the yeast surface display system. Recent results in our lab have shown that the efficiency with which a single-chain TCR is displayed on the yeast surface is correlated with its secretion efficiency. By screening for BPTI **mutants** which display well on yeast, we hope to engineer a fusion partner which will promote highly efficient secretion of **endostatin**.

L1 ANSWER 3 OF 7 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:767829 CAPLUS
DOCUMENT NUMBER: 132:89706
TITLE: Structural basis and potential role of heparin/heparan sulfate binding to the angiogenesis inhibitor endostatin
AUTHOR(S): Sasaki, Takako; Larsson, Helena; Kreuger, Johan; Salmivirta, Markku; Claesson-Welsh, Lena; Lindahl, Ulf; Hohenester, Erhard; Timpl, Rupert
CORPORATE SOURCE: Max-Planck-Institut fur Biochemie, Martinsried, D-82152, Germany
SOURCE: EMBO J. (1999), 18(22), 6240-6248
CODEN: EMJODG; ISSN: 0261-4189
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Recombinant mouse endostatin produced by mammalian cells was shown to bind to heparin with a Kd of 0.3 .mu.M, suggesting that this interaction may play a role in its anti-angiogenic activity. Alanine mutagenesis demonstrated that a major site of four clustered arginines (positions 155, 158, 184 and 270) and a second site (R193,R194) are essential for binding. The same epitopes also participate in endostatin binding to heparan sulfate and sulfatides but not in its binding to the extracellular protein ligands fibulin-1 and fibulin-2. Analyses with various heparin fragments demonstrated a min. size (12mer) for efficient binding to endostatin and a crucial role of 2-O- and 6-O-sulfation. Furthermore, a substantial proportion (10-50%) of heparan sulfate chains obtained from various tissues showed a distinct binding to endostatin, indicating its potential to interact with extracellular and/or membrane-bound proteoglycans. Angiogenesis induced by basic fibroblast growth factor-2 (FGF-2), but not by vascular endothelial growth factor (VEGF), in a chick chorioallantoic membrane assay could be inhibited by endostatin in a dose-dependent manner. The **mutational** block of heparin binding decreased **endostatin** inhibition to low levels but elimination of zinc binding had no effect.
REFERENCE COUNT: 65
REFERENCE(S): (1) Andac, Z; J Mol Biol 1999, V287, P253 CAPLUS
(2) Beck, L; FASEB J 1997, V11, P365 CAPLUS
(4) Boehm, T; Biochem Biophys Res Commun 1998, V252, P190 CAPLUS
(5) Boehm, T; Nature 1997, V390, P404 CAPLUS
(6) Brooks, P; Science 1994, V264, P569 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L1 ANSWER 4 OF 7 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:388288 CAPLUS
DOCUMENT NUMBER: 131:39759

TITLE: Restin and apomigren fragments of human collagen type XV .alpha.1 chain and their anti-angiogenic activities

INVENTOR(S): Sukhatme, Vikas P.
PATENT ASSIGNEE(S): Beth Israel Deaconess Medical Center, USA

SOURCE: PCT Int. Appl., 94 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9929856	A1	19990617	WO 1998-US26058	19981208
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, US, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9918088	A1	19990628	AU 1999-18088	19981208
EP 1037985	A1	20000927	EP 1998-962966	19981208
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				

PRIORITY APPLN. INFO.:
US 1997-67888 19971208
US 1998-82663 19980422
US 1998-108536 19981116
WO 1998-US26058 19981208

AB The invention relates to restin, a novel anti-angiogenic protein is described, as well as its fragment, designated apomigren. Restin is a proteolytic fragment of the C-terminal fragment of the NC10 domain of the .alpha.1 chain of human collagen type XV. Apomigren is a fragment of restin, and comprises the C-terminal 85 residues of restin,. Methods for expression of the proteins at high titer are also described. Restin inhibits the migration of endothelial cells in vitro and suppresses the growth of tumors in a xenograft renal carcinoma model. Apomigren has anti-angiogenic activity equal or superior to that of endostatin.

REFERENCE COUNT: 6
REFERENCE(S):
(1) Bachelot; Proceedings of the 89th Annual Meeting of the American Association for Cancer Research 1998, V39, P271
(2) Childrens Medical Center; WO 9715666 A 1997 CAPLUS
(3) Ramchandran, R; Biochem Biophys Res Comm

1999, V255, P735 CAPLUS
(4) Rehn, M; J Biol Chem 1994, V269(19), P13929 CAPLUS
(6) Searle, G; WO 9916899 A 1999 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L1 ANSWER 5 OF 7 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:388287 CAPLUS
DOCUMENT NUMBER: 131:41277
TITLE: **Mutants** of **endostatin**, "em 1" having anti-angiogenic activity and methods of use thereof
INVENTOR(S): Sukhatme, Vikas P.
PATENT ASSIGNEE(S): Beth Israel Deaconess Medical Center, USA
SOURCE: PCT Int. Appl., 105 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9929855	A1	19990617	WO 1998-US26057	19981208
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, US, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9917180	A1	19990628	AU 1999-17180	19981208
EP 1037983	A1	20000927	EP 1998-962006	19981208
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				

PRIORITY APPLN. INFO.:
US 1997-67888 19971208
US 1998-82663 19980422
US 1998-108536 19981116
WO 1998-US26057 19981208

AB Described herein are novel **mutants** of **endostatin**, one of which, designated "EM 1", has anti-angiogenic activity similar or superior to that of wild type **endostatin**. The invention relates to the discovery of an isolated anti-angiogenic peptide, wherein the C-terminal end of the peptide comprises the amino acid sequence SYIVLCIE, which has anti-angiogenic properties. Designated "EM 1", this protein comprises a **mutated endostatin** protein, where the **mutation** comprises a

deletion of nine consecutive amino acids from the C-terminus of the **mutated endostatin** protein (e.g., NSFMTSFSK). EM 1 terminates in the amino acid sequence SYIVLCIE. The invention also comprises isolated polynucleotides encoding EM 1, operably linked to expression sequence, and host cells transformed with such a construct. Antibodies to EM 1 are also disclosed. The invention also relates to processes for producing EM 1, fusion proteins contg. EM 1, and compns. comprising EM 1 or fusion products thereof. The invention also discloses methods of producing polypeptides encoding EM 1.

REFERENCE COUNT: 8

REFERENCE(S):
(1) Boehm, T; Biochemical and Biophysical Research Communications 1998, V252, P190 CAPLUS
(2) Dhanabal, M; Cancer Research 1999, V59, P189 CAPLUS
(3) Ding, Y; Proc Natl Acad Sci USA 1998, V95, P10443 CAPLUS
(5) Hohenester, E; The EMBO Journal 1998, V17(6), P1656 CAPLUS
(7) O'Reilly, M; Cell 1997, V88(2), P277 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L1 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1999:66274 CAPLUS
DOCUMENT NUMBER: 130:246461

TITLE: **Endostatin**: yeast production, **mutants**, and antitumor effect in renal cell carcinoma

AUTHOR(S): Dhanabal, Mohanraj; Ramchandran, Ramani; Volk, Ruediger; Stillman, Saac E.; Lombardo, Michelle; Iruela-Arispe, M. L.; Simons, Michael; Sukhatme, Vikas P.

CORPORATE SOURCE: Renal and Cardiology Divisions, Departments of Medicine and Pathology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, 02215, USA

SOURCE: Cancer Res. (1999), 59(1), 189-197
CODEN: CNREA8; ISSN: 0008-5472

PUBLISHER: AACR Subscription Office
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Endostatin is a Mr 20,000 COOH-terminal fragment of collagen XVIII that inhibits the growth of several primary tumors. We report here the cloning and expression of mouse endostatin in both prokaryotic and eukaryotic expression systems. Sol. recombinant protein expressed in yeast (15-20 mg/L) inhibited the proliferation and migration of endothelial cells in response to stimulation by basic fibroblast growth factor. A rabbit polyclonal antibody was raised

that showed pos. immunoreactivity to the recombinant protein expressed from both systems. Importantly, the biol. activity of the mouse recombinant protein could be neutralized by this antiserum in both endothelial proliferation and chorioallantoic membrane assays. Systemic administration of endostatin at 10 mg/kg suppressed the growth of renal cell cancer in a nude mouse model. The inhibition of tumor growth with sol. yeast-produced protein was comparable to that obtained with non-refolded pptd. protein ex- pressed from bacteria. In addn., two closely related COOH-terminal deletion **mutants** of **endostatin** were also tested and showed strikingly differing activity. Collectively, these findings demonstrate the expression of a biol. active form of mouse **endostatin** in yeast, define a role for the mol. in inhibiting endothelial cell migration, extend its antitumor effects to renal cell carcinoma, and provide a formal proof (via the neutralizing antiserum expts. and the **mutant** data) that **endostatin** (and not a possible contaminant) acts as an antiangiogenic agent. Finally, the high level expression of mouse endostatin in yeast serves as an endotoxin free, sol. source of protein for fundamental studies on the mechanisms of tumor growth suppression by angiogenesis inhibitors.

REFERENCE COUNT: 43

REFERENCE(S):
(1) Angiolillo, A; J Exp Med 1995, V182, P155 CAPLUS
(4) Boehm, T; Nature (Lond) 1997, V390, P404 CAPLUS
(5) Brooks, P; Cell 1998, V92, P391 CAPLUS
(6) Burrows, F; Pharmacol Ther 1994, V64, P155 CAPLUS
(8) Dhanabal, M; J Immunol Methods 1995, V182, P165 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L1 ANSWER 7 OF 7 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1998:746502 CAPLUS

DOCUMENT NUMBER: 130:76561

TITLE: Zinc-binding of endostatin is essential for its antiangiogenic activity

AUTHOR(S): Boehm, Thomas; O'Reilly, Michael S.; Keough, Karen; Shiloach, Joseph; Shapiro, Robert; Folkman, Judah

CORPORATE SOURCE: Department of Surgery, Departments of Surgery and Cellular Biology, Harvard Medical School, The Children's Hospital, Boston, MA, 02115, USA

SOURCE: Biochem. Biophys. Res. Commun. (1998), 252(1), 190-194
CODEN: BBRCA9; ISSN: 0006-291X

PUBLISHER: Academic Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Endostatin is a potent angiogenesis inhibitor in vitro and in vivo. We used the yeast Pichia pastoris to express and purify sol. endostatin. It was discovered that metal chelating agents can induce N-terminal degrdn. of endostatin. We theorized that a metal was removed from endostatin which changed the conformation and allowed a contaminating protease to degrade the N-terminus. At. absorption and amino acid anal. of endostatin purified from Pichia pastoris and mammalian cells showed a 1:1 molar ratio of Zn2+ to protein. H-Y. Ding et al. (1998)have shown that histidines 1, 3, 11, and aspartic acid 76 coordinate the Zn2+ atom (1). An H1/3A double, an H11A, and a D76A single **mutant** of **endostatin** were not able to regress Lewis lung carcinoma. We conclude that the ability of endostatin to bind Zn2+ is essential for its antiangiogenic activity. (c) 1998 Academic Press.

REFERENCE COUNT: 13

REFERENCE(S):
(1) Berg, J; Science 1996, V271, P1081 CAPLUS
(2) Boehm, T; Nature 1997, V390, P404 CAPLUS
(3) Coleman, J; Annu Rev Biochem 1992, V61, P897 CAPLUS
(4) Cunningham, B; Science 1990, V250, P1709 CAPLUS
(5) Cunningham, B; Science 1991, V253, P545 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO' ENTERED AT 14:32:43 ON 06 APR 2001)

L2 38 S L1
L3 14 DUP REM L2 (24 DUPLICATES REMOVED)

L3 ANSWER 1 OF 14 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 1

ACCESSION NUMBER: 2001:140572 BIOSIS
DOCUMENT NUMBER: PREV200100140572
TITLE: Lack of type XV collagen causes a skeletal myopathy and cardiovascular defects in mice.
AUTHOR(S): Eklund, Lauri; Piuhola, Jarkko; Komulainen, Jyrki; Sormunen, Raija; Ongvarrasopone, Chalermporn; Fassler, Reinhard; Muona, Anu; Ilves, Mika; Ruskoaho, Heikki; Takala, Timo E. S.; Pihlajaniemi, Taina (1)
CORPORATE SOURCE: (1) Collagen Research Unit, Biocenter Oulu, and Departments of Medical Biochemistry, University of Oulu, 90014, Oulu: taina.pihlajaniemi@oulu.fi Finland
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (January 30, 2001) Vol. 98, No. 3, pp. 1194-1199. print.
ISSN: 0027-8424.
DOCUMENT TYPE: Article
LANGUAGE: English

SUMMARY LANGUAGE: English

AB Type XV collagen occurs widely in the basement membrane zones of tissues, but its function is unknown. To understand the biological role of this protein, a null **mutation** in the Col15a1 gene was introduced into the germ line of mice. Despite the complete lack of type XV collagen, the **mutant** mice developed and reproduced normally, and they were indistinguishable from their wild-type littermates. However, Col15a1-deficient mice showed progressive histological changes characteristic for muscular diseases after 3 months of age, and they were more vulnerable than controls to exercise-induced muscle injury. Despite the antiangiogenic role of type XV collagen-derived **endostatin**, the development of the vasculature appeared normal in the null mice. Nevertheless, ultrastructural analyses revealed collapsed capillaries and endothelial cell degeneration in the heart and skeletal muscle. Furthermore, perfused hearts showed a diminished inotropic response, and exercise resulted in cardiac injury, changes that mimic early or mild heart disease. Thus, type XV collagen appears to function as a structural component needed to stabilize skeletal muscle cells and microvessels.

L3 ANSWER 2 OF 14 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 2001:233100 SCISEARCH
THE GENUINE ARTICLE: 411AZ
TITLE: Collagens and collagen-related diseases
AUTHOR: Myllyharju J; Kivirikko K I (Reprint)
CORPORATE SOURCE: Univ Oulu, Dept Med Biochem, POB 5000, Oulu 90014, Finland (Reprint); Univ Oulu, Dept Med Biochem, Oulu 90014, Finland; Univ Oulu, Bioctr, Collagen Res Unit, Oulu, Finland
COUNTRY OF AUTHOR: Finland
SOURCE: ANNALS OF MEDICINE, (FEB 2001) Vol. 33, No. 1, pp. 7-21.
Publisher: ROYAL SOC MEDICINE PRESS LTD, 1 WIMPOLE STREET, LONDON W1M 8AE, ENGLAND.
ISSN: 0785-3890.
DOCUMENT TYPE: General Review; Journal
LANGUAGE: English
REFERENCE COUNT: 144

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The collagen superfamily of proteins plays a dominant role in maintaining the integrity of various tissues and also has a number of other important functions. The superfamily now includes more than 20 collagen types with altogether at least 38 distinct polypeptide chains, and more than 15 additional proteins that have collagen-like domains. Most collagens form polymeric assemblies, such as fibrils, networks and filaments, and the superfamily can be divided into several families based on these assemblies and other features. All collagens also contain noncollagenous domains, and many of these

have important functions that are distinct from those of the collagen domains, Major interest has been focused on **endostatin**, a fragment released from type XVIII collagen. which potently inhibits angiogenesis and tumour growth. Collagen synthesis requires eight specific post-translational enzymes, some of which are attractive targets for the development of drugs to inhibit collagen accumulation in fibrotic diseases. The critical roles of collagens have been clearly illustrated by the wide spectrum of diseases caused by the more than 1000 **mutations** that have thus far been identified in 22 genes for 12 out of the more than 20 collagen types. These diseases include osteogenesis imperfecta, many chondrodysplasias, several subtypes of the Ehlers-Danlos syndrome, Alport syndrome, Bethlem myopathy, certain subtypes of epidermolysis bullosa, Knobloch syndrome and also some cases of osteoporosis, arterial aneurysms. osteoarthrosis, and intervertebral disc disease, The characterization of **mutations** in additional collagen genes will probably add further diseases to this list. Mice with genetically engineered collagen **mutations** have proved valuable for defining the functions of various collagens and for studying many aspects of the related diseases.

L3 ANSWER 3 OF 14 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2001-091568 [10] WPIDS
DOC. NO. CPI: C2001-027024
TITLE: Designing metal ion for molecular dynamics simulation, useful e.g. for drug design by energy refinement of zinc-binding protein, maintains correct polyhedral geometry.
DERWENT CLASS: B04 D16
INVENTOR(S): PANG, Y
PATENT ASSIGNEE(S): (MAYO-N) MAYO FOUND MEDICAL EDUCATION & RES
COUNTRY COUNT: 91
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000078938	A1	20001228	(200110)*	EN	42

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE

WO 2000078938 A1 WO 2000-US16599 20000616

PRIORITY APPLN. INFO: US 1999-139845 19990618
AN 2001-091568 [10] WPIDS
AB WO 200078938 A UPAB: 20010220
NOVELTY - Method for designing a metal ion (I) for use in molecular dynamics (MD) simulations comprises (i) building metal ion molecule (II), with polyhedral geometry, having a central atom (CA) and a dummy atom (DA), covalently bonded together; (ii) assigning a van der Waals radius (r) to CA and (iii) assigning a charge to DA.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) performing at least nanosecond long MD simulations by assigning force field parameters; and

(2) a simulated (II) comprising CA with r greater than zero, but zero charge, covalently linked to one or more DA with r about zero, with the overall charge of (II) being distributed evenly over DA.

USE - The method is particularly used in MD simulations involving metalloproteins, for e.g. computer-aided protein-ligand docking simulations, energy refinement of e.g. zinc-binding proteins, stimulating charge-energy transfer of transition metal ions, pharmaceutical development (typical examples: design of improved **endostatin** mimics, zinc-finger **mutants**, phosphodiesterase **mutants**, inhibitors of anthrax and botulinum toxins and angiogenesis inhibitors for cancer treatment) and design of transcription factors for gene therapy and for (in)organic molecule simulation.

ADVANTAGE - The method imposes the proper orientational requirements for the coordinating ligand, maintains the polyhedral geometry of the coordination complex during MD simulations (contrast the non-bonded model) and can simulate charge-transfer effects in both transition and main group metals, including exchange of ambidentate ligands. The method, and new force field parameters for (II), provide excellent agreement between X-ray crystallographical analysis and 2 nanosecond (ns) MD simulations of zinc-binding proteins and a better understanding of metal-ligand coordination; allow precise evaluation of thermodynamic parameters, and also refinement of X-ray structures of metal-coordinating proteins where an electron density map does not indicate which oxygen of a carboxylate group is coordinated to the metal.

Dwg.0/7

L3 ANSWER 4 OF 14 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2000-687317 [67] WPIDS
DOC. NO. CPI: C2000-209206
TITLE: Immunogenic composition for the treatment and diagnosis of cancer comprises an anti-VEGF

(vascular endothelial growth factor) antibody binding the same epitope as the monoclonal antibody ATCC PTA 1595.

DERWENT CLASS: B04 B05 D16
INVENTOR(S): BREKKEN, R A; THORPE, P E
PATENT ASSIGNEE(S): (TEXA) UNIV TEXAS SYSTEM
COUNTRY COUNT: 91
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000064946	A2	20001102	(200067)*	EN	93
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW					
AU 2000048049	A	20001110	(200109)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000064946	A2	WO 2000-US11367	20000428
AU 2000048049	A	AU 2000-48049	20000428

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2000048049	A Based on	WO 200064946

PRIORITY APPLN. INFO: US 1999-131432 19990428
AN 2000-687317 [67] WPIDS
AB WO 200064946 A UPAB: 20001223
NOVELTY - A composition (I) comprising a biologically effective amount of an anti-VEGF (vascular endothelial growth factor) antibody or antigen binding fragment that binds to substantially to the same epitope as the monoclonal antibody ATCC PTA 1595, is new.

DETAILED DESCRIPTION - A composition (I) comprising a biologically effective amount of an anti-VEGF antibody or antigen binding fragment that binds to substantially to the same epitope as the monoclonal antibody ATCC PTA 1595 and which significantly inhibits VEGF binding to the VEGF receptor VEGFR2 (KDR/Flk-1) without inhibiting VEGF binding to the VEGF receptor VEGFR1 (Flt-1).

INDEPENDENT CLAIMS are also included for the following:

(1) a composition (II) comprising a biologically effective amount of an anti-VEGF antibody or antigen binding fragment that

binds to substantially to the same epitope as the monoclonal antibody 2C3 (ATCC PTA 1595) for use in inhibiting angiogenesis without substantial inhibition of macrophages, osteoclasts or chondroclasts;

(2) a kit comprising (I);

(3) a hybridoma producing the monoclonal antibody in (I);

(4) monoclonal antibody ATCC PTA 1595;

(5) a method for preparing an anti-VEGF antibody or antigen binding fragment that binds to substantially to the same epitope as the monoclonal antibody ATCC PTA 1595 comprising immunizing a non-human animal with an immunizing composition comprising at least a first immunogenic VEGF component and selecting from the immunized animal an antibody that substantially cross-reacts with ATCC PTA 1595;

(6) a method of detecting VEGF comprising contacting a composition suspected of containing VEGF with (I) allowing formation of a VEGF/antibody complex and detecting the complex;

(7) a method of inhibiting VEGF binding to the VEGF receptor VEGFR2 without significantly inhibiting VBGF to the VEGF receptor VEGFR1 comprising contacting a homo- or heterogeneous population of cells that express VEGFR2 (KDR-Flk-1) and VEGFR1 (Flt-1) with a biologically effective amount of (I);

(8) a method for specifically inhibiting VEGF-induced endothelial cell proliferation comprising contacting a population of endothelial cells with a biologically effective amount of (I);

(9) a method for specifically inhibiting VEGF-induced endothelial cell proliferation without significantly inhibiting VEGF-stimulated macrophage, osteoclast or chondroclast function comprising contacting a tissue containing endothelial cells and at least one of macrophages, osteoclasts or chondroclasts with a biologically effective amount of (I);

(10) a method of inhibiting angiogenesis comprising contacting a population of potentially angiogenic blood vessels with an anti-angiogenic composition comprising a biologically effective amount of (I);

(11) a method for treating an angiogenic disease comprising administering to an animal with an angiogenic disease at least a first pharmaceutical composition comprising a therapeutically effective amount of (I);

(12) a method for delivering a diagnostic or therapeutic agent to a vascularized tumor comprising administering to an animal with a vascularized tumor a biologically effective amount of (I);

(13) a method for treating cancer comprising administering at least a first pharmaceutical composition to an animal that has, or is at risk of developing a vascularized solid tumor, a metastatic tumor or metastases from a primary tumor where the first pharmaceutical composition is (I); and

(14) a method for treating cancer comprising:

(i) administering (I) to an animal that has a vascularized

solid tumor, a metastatic tumor or metastases from a primary tumor, localizing the antibody of the composition to the tumor vasculature or stroma; and

(ii) subsequently administering to the animal a second composition that comprises a substantially inactive prodrug that is cleaves by the biological agent attached to the antibody in the first composition, releasing a substantially active drug within the tumor vasculature or stroma.

ACTIVITY - Cytostatic; antiproliferative.

USE - The composition is useful for the treatment and diagnosis of cancer, especially vascularized solid tumors. It is also useful in the manufacture of a medicament for treating cancer by inhibiting VEGF binding to the VEGF receptor VEGFR2 (KDR/Flk-1) without inhibiting VEGF binding to the VEGF receptor VEGFR1 (Flt-1) (claimed). The composition may also be used to detect VEGF in a sample.

Dwg.4/4

L3 ANSWER 5 OF 14 MEDLINE DUPLICATE 2

ACCESSION NUMBER: 2000287377 MEDLINE
DOCUMENT NUMBER: 20287377
TITLE: Endostatin-induced tyrosine kinase signaling through the Shb adaptor protein regulates endothelial cell apoptosis.
AUTHOR: Dixelius J; Larsson H; Sasaki T; Holmqvist K; Lu L; Engstrom A; Timpl R; Welsh M; Claesson-Welsh L
CORPORATE SOURCE: Department of Genetics and Pathology, Rudbeck Laboratory, Uppsala, Sweden.
SOURCE: BLOOD, (2000 Jun 1) 95 (11) 3403-11.
Journal code: A8G. ISSN: 0006-4971.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals; Cancer Journals
ENTRY MONTH: 200009
ENTRY WEEK: 20000901

AB **Endostatin**, which corresponds to the C-terminal fragment of collagen XVIII, is a potent inhibitor of angiogenesis. Fibroblast growth factor-2 (FGF-2)-induced angiogenesis in the chicken chorioallantoic membrane was inhibited by **endostatin**, but not by an **endostatin mutant** R158/270A, lacking heparin-binding ability. **Endostatin** was internalized by endothelial cells, but not by mouse fibroblasts. Treatment of murine brain endothelial (IBE) cells with **endostatin** reduced the proportion of cells in S phase, whereas growth-arrested IBE cells in collagen gels treated with **endostatin** displayed enhanced tubular morphogenesis. IBE cells overexpressing Shb, an adaptor protein implicated in angiostatin-induced apoptosis, displayed

elevated apoptosis and decreased tubular morphogenesis in collagen gels in response to **endostatin** when added together with FGF-2. Induction of apoptosis was dependent on the heparin-binding ability of **endostatin** and the expression of Shb with a functional Src homology 2 (SH2)-domain. **Endostatin** treatment for 10 minutes or 24 hours induced tyrosine phosphorylation of Shb and formation of multiprotein complexes. An Shb SH2 domain fusion protein precipitated a 125-kd phosphotyrosyl protein in **endostatin**-treated cells. The 125-kd component either contained intrinsic tyrosine kinase activity or occurred in complex with a tyrosine kinase. In conclusion, our data show that **endostatin** induces tyrosine kinase activity and enhanced apoptosis in FGF-treated endothelial cells.

L3 ANSWER 6 OF 14 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 2000171580 MEDLINE
DOCUMENT NUMBER: 20171580
TITLE: Variable zinc coordination in endostatin.
AUTHOR: Hohenester E; Sasaki T; Mann K; Timpl R
CORPORATE SOURCE: Biophysics Section, Blackett Laboratory, Imperial College, London, SW7 2AZ, UK.. hohenester@ic.ac.uk
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (2000 Mar 17) 297 (1) 1-6.
Journal code: J6V. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 200006
ENTRY WEEK: 20000603
AB **Endostatin** is a proteolytic fragment of collagen XVIII that potently inhibits angiogenesis and tumour growth. Human **endostatin** contains a zinc ion, bound near the N terminus, which was not observed in the original structure of mouse **endostatin** at pH 5. Controversial data exist on the role of this zinc ion in the anti-tumour activity. We report two new crystal structures of mouse **endostatin** at pH 8.5 with bound zinc. One crystal form shows a metal ion coordination similar to that in human **endostatin** (His132, His134, His142, Asp207), but the conformation of the N-terminal segment is different. In the other crystal form, Asp136 replaces His132 as a zinc ligand. Site-directed **mutagenesis** of zinc-binding residues demonstrates that both coordination geometries occur in solution. The large degree of structural heterogeneity of the zinc-binding site has implications for **endostatin** function. We conclude that zinc is likely to play a structural rather than a critical functional role in **endostatin**. Copyright 2000 Academic Press.

L3 ANSWER 7 OF 14 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
Searcher : Shears 308-4994

ACCESSION NUMBER: 1999-404943 [34] WPIDS
CROSS REFERENCE: 1999-385604 [32]; 1999-394974 [33]
DOC. NO. CPI: C1999-119491
TITLE: Production of anti-angiogenic proteins.
DERWENT CLASS: B04 D16
INVENTOR(S): SUKHATME, V P
PATENT ASSIGNEE(S): (BETH-N) BETH ISRAEL DEACONESS MEDICAL CENT
COUNTRY COUNT: 85
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9929878	A2	19990617	(199934)*	EN	96
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW					
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW					
AU 9918065	A	19990628	(199946)		
EP 1038011	A2	20000927	(200048)	EN	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9929878	A2	WO 1998-US25892	19981208
AU 9918065	A	AU 1999-18065	19981208
EP 1038011	A2	EP 1998-962932	19981208
		WO 1998-US25892	19981208

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9918065	A	Based on	WO 9929878
EP 1038011	A2	Based on	WO 9929878

PRIORITY APPLN. INFO: US 1998-108536 19981116; US 1997-67888 19971208; US 1998-82663 19980422

AN 1999-404943 [34] WPIDS
CR 1999-385604 [32]; 1999-394974 [33]
AB WO 9929878 A UPAB: 20001001
NOVELTY - Production of anti-angiogenic proteins particularly angiostatin, endostatin or restin by using a recombinant yeast expression system, particularly Pichia pastoris host cells is new.
DETAILED DESCRIPTION - (A) A novel method of producing a biologically active anti-angiogenic protein (AAP) or a biologically

active mutant, fragment, derivative, or fusion protein (FP) comprises:

(a) inserting an isolated polynucleotide (PN) comprising a PN sequence encoding an AAP, or a mutant, derivative, fragment or FP, into a yeast expression vector, where the vector contains a multiple cloning site; and

(b) transforming an appropriate yeast strain with a vector as in (a) and maintaining the yeast strain under conditions for the production of the AAP to produce a biologically active AAP, or mutant, derivative, fragment or FP.

INDEPENDENT CLAIMS are also included for the following:

(1) a polypeptide encoding an AAP where the AAP, **mutant** , derivative, fragment or FP is selected from **endostatin**, angiostatin or restin or any **mutants**, derivatives, fragments or FPs, or any combination;

(2) a biologically active AAP, mutant, derivative, fragment or FP produced by a method as in (A);

(3) a method of producing a biologically active AAP, or a biologically active mutant, fragment, derivative, or FP comprising:

(a) inserting an isolated PN comprising a PN sequence encoding an AAP, or a mutant, derivative, fragment or FP, where the PN additionally comprises a linker, where the PN linker encodes at least one amino acid, into a yeast expression vector comprising a pPICz alpha A plasmid, where the plasmid contains a multiple cloning site; and

(b) transforming a Pichia pastoris yeast strain with a vector of (a) and maintaining the yeast strain for the production of the AAP comprising at least one amino acid residue resulting from the linker PN, to produce a biologically active AAP, or a mutant, derivative, fragment or FP;

(4) a biologically active AAP produced by a method as in (3);

(5) a producing a biologically active AAP, or a biologically active mutant, fragment, derivative or FP comprising:

(a) inserting an isolated PN comprising a PN sequence encoding an AAP, or a mutant, derivative, fragment or FP, where the PN additionally comprises a linker, where the PN linker encodes at least one amino acid, into a yeast expression vector comprising a pPICz alpha A plasmid where the plasmid contains a multiple cloning site and where the cloning site additionally comprises a histidine tag motif; and

(b) transforming a P. pastoris yeast strain with a vector as in (a) and maintaining the yeast strain for the production of the AAP comprising at least one amino acid residue resulting from the linker PN, and where the protein additionally comprises a histidine tag motif, to produce a biologically active AAP, or a mutant, derivative, fragment or FP;

(6) a biologically active AAP produced by a method as in (5); and

(7) a method as in (3) or (5) where the PN encodes angiostatin,

endostatin, restin or **mutants**, derivatives, fragments or FPs, or any combinations.

USE - The AAP, mutant, derivative, fragment or FP can be used to inhibit undesirable angiogenesis in a mammal (claimed). They can be used for inhibition of endothelial activity such as endothelial cell migration, inhibition of tumor growth, arrest of endothelial cells in G1 phase of the cell cycle, and inducing apoptosis in endothelial cells. They can be used for treating e.g. angiogenesis-dependent cancers and tumors, tumor metastasis, benign tumors e.g. hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas, rheumatoid arthritis, psoriasis, ocular angiogenic diseases e.g. diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, Osler-Webber syndrome, myocardial angiogenesis, plaque neovascularization, telangiectasia, hemophiliac joints, angiofibroma, wound granulation, intestinal adhesions, Crohn's disease, atherosclerosis, scleroderma, hypertrophic scars i.e. keloids, or cat scratch disease and ulcers, as a birth control agent by preventing vascularization required for embryo implantation. They can also be used for the production of antibodies.

ADVANTAGE - Using the methods, the AAPs can be produced in high yields, e.g. 10-20mg/l of culture medium and retain high biological activity.

Dwg.0/27

L3 ANSWER 8 OF 14 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD

ACCESSION NUMBER: 1999-385604 [32] WPIDS
CROSS REFERENCE: 1999-394974 [33]; 1999-404943 [34]
DOC. NO. CPI: C1999-113510
TITLE: **Mutant endostatin** having anti-angiogenic activity.
DERWENT CLASS: B04 D16
INVENTOR(S): SUKHATME, V P
PATENT ASSIGNEE(S): (BETH-N) BETH ISRAEL DEACONESS MEDICAL CENT
COUNTRY COUNT: 85
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9929855	A1	19990617	(199932)*	EN	105
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW					
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW					
AU 9917180	A	19990628	(199946)		
EP 1037983	A1	20000927	(200048)	EN	

R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9929855	A1	WO 1998-US26057	19981208
AU 9917180	A	AU 1999-17180	19981208
EP 1037983	A1	EP 1998-962006	19981208
		WO 1998-US26057	19981208

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9917180	A	Based on	WO 9929855
EP 1037983	A1	Based on	WO 9929855

PRIORITY APPLN. INFO: US 1998-108536 19981116; US 1997-67888 19971208; US 1998-82663 19980422

AN 1999-385604 [32] WPIDS

CR 1999-394974 [33]; 1999-404943 [34]

AB WO 9929855 A UPAB: 20001001

NOVELTY - A **mutant endostatin** (EM) having anti-angiogenic activity comprising a C-terminal sequence (I), is new.

DETAILED DESCRIPTION - An isolated anti-angiogenic peptide, where the C-terminal comprises the amino acid sequence SYIVLCIE (I).

INDEPENDENT CLAIMS are also included for the following:

(a) an isolated polynucleotide amplified by the following primers (P1), and (P2):

TTCCATATGCATACTCATCAGGACTTTCAGGCA (P1); and

TTAGCGGCCGCCTACTCAATGCAGAGGACGATGTA (P2);

(b) a host cell transformed with a polynucleotide, encoding EM1, operably linked to an expression control sequence;

(c) production of EM1;

(d) a fusion protein comprising two or more proteins and also comprising EM1;

(e) a process for providing a mammal with EM1;

(f) producing an isolated polynucleotide which hybridizes under moderate stringency;

(g) an EM1 polynucleotide isolated by (f);

(h) antibodies to EM1; and

(i) a mutant, derivative, analogue or homologue of EM1.

ACTIVITY - Anti-angiogenic; cytostatic.

MECHANISM OF ACTION - None given.

USE - Compositions comprising EM1 or fusion proteins comprising EM1, are useful for treating diseases characterized by angiogenic activity, such as angiogenesis-dependent cancers, benign tumors,

rheumatoid arthritis, psoriasis, ocular angiogenesis, Osler-Webber Syndrome, myocardial angiogenesis, plaque neovascularization, telangiectasia, hemophiliac joints, angiofibroma, wound granulation, intestinal adhesions, atherosclerosis, scleroderma, hypertrophic scars, cat scratch disease, Helicobacter pylori ulcers, dialysis graft vascular access stenosis, contraception and obesity. In particular, the diseases treatable by EM1 comprise cancer, especially renal cancer. The methods provide a means for introducing EM1 into mammalian cells via gene therapy, for production of EM1 via recombinant means, as well as recombinant production of the EM1 protein. (All claimed).

ADVANTAGE - EM1 performs as well or better than whole endostatin. In a nude mouse model, growth of renal cell cancer (RCC) was suppressed by systemic administration of EM1 at a rate of 20 mg/kg body weight. Use of EM1 is advantageous for treatment of angiogenic diseases in that increasingly smaller peptides are more potent on a weight basis, and may be able to better penetrate tissues.

Dwg.20/26

L3 ANSWER 9 OF 14 MEDLINE DUPLICATE 4

ACCESSION NUMBER: 2000031635 MEDLINE
DOCUMENT NUMBER: 20031635
TITLE: Structural basis and potential role of heparin/heparan sulfate binding to the angiogenesis inhibitor endostatin.
AUTHOR: Sasaki T; Larsson H; Kreuger J; Salmivirta M; Claesson-Welsh L; Lindahl U; Hohenester E; Timpl R
CORPORATE SOURCE: Max-Planck-Institut fur Biochemie, Am Klopferspitz 18A, D-82152 Martinsried, Germany.
SOURCE: EMBO JOURNAL, (1999 Nov 15) 18 (22) 6240-8. Journal code: EMB. ISSN: 0261-4189.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200003
ENTRY WEEK: 20000304

AB Recombinant mouse **endostatin** produced by mammalian cells was shown to bind to heparin with a K(d) of 0.3 microM, suggesting that this interaction may play a role in its anti-angiogenic activity. Alanine **mutagenesis** demonstrated that a major site of four clustered arginines (positions 155, 158, 184 and 270) and a second site (R193, R194) are essential for binding. The same epitopes also participate in **endostatin** binding to heparan sulfate and sulfatides but not in its binding to the extracellular protein ligands fibulin-1 and fibulin-2. Analyses with various heparin fragments demonstrated a minimum size (12mer) for efficient binding to **endostatin** and a crucial role of 2-O- and

6-O-sulfation. Furthermore, a substantial proportion (10-50%) of heparan sulfate chains obtained from various tissues showed a distinct binding to **endostatin**, indicating its potential to interact with extracellular and/or membrane-bound proteoglycans. Angiogenesis induced by basic fibroblast growth factor-2 (FGF-2), but not by vascular endothelial growth factor (VEGF), in a chick chorioallantoic membrane assay could be inhibited by **endostatin** in a dose-dependent manner. The **mutational** block of heparin binding decreased **endostatin** inhibition to low levels but elimination of zinc binding had no effect.

L3 ANSWER 10 OF 14 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 1999380158 MEDLINE
DOCUMENT NUMBER: 99380158
TITLE: Endostatin inhibits VEGF-induced endothelial cell migration and tumor growth independently of zinc binding.
AUTHOR: Yamaguchi N; Anand-Apte B; Lee M; Sasaki T; Fukai N; Shapiro R; Que I; Lowik C; Timpl R; Olsen B R
CORPORATE SOURCE: Department of Cell Biology, Harvard Medical School, Boston, MA 02115, USA.
CONTRACT NUMBER: AR36820 (NIAMS)
EY12109 (NEI)
SOURCE: EMBO JOURNAL, (1999 Aug 16) 18 (16) 4414-23.
Journal code: EMB. ISSN: 0261-4189.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199912
ENTRY WEEK: 19991201
AB **Endostatin**, produced as recombinant protein in human 293-EBNA cells, inhibits the migration of human umbilical vein endothelial cells (HUVECs) in response to vascular endothelial growth factor (VEGF) in a dose-dependent manner and prevents the subcutaneous growth of human renal cell carcinomas in nude mice at concentrations and in doses that are from 1000- to 100 000-fold lower than those previously reported. The inhibition of migration is not affected by **mutations** which eliminate Zn or heparin binding and inhibition of tumor growth does not depend on Zn binding. The results of the migration assays suggest that **endostatin** causes a block at one or more steps in VEGF-induced migration, while VEGF in turn can cause a block of the inhibition by **endostatin** of VEGF-induced migration of HUVECs.

L3 ANSWER 11 OF 14 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 1999107224 MEDLINE

DOCUMENT NUMBER: 99107224
TITLE: **Endostatin**: yeast production, **mutants**, and antitumor effect in renal cell carcinoma.
AUTHOR: Dhanabal M; Ramchandran R; Volk R; Stillman I E; Lombardo M; Iruela-Arispe M L; Simons M; Sukhatme V P
CORPORATE SOURCE: Renal Division, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts 02215, USA.
SOURCE: CANCER RESEARCH, (1999 Jan 1) 59 (1) 189-97. Journal code: CNF. ISSN: 0008-5472.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199904
ENTRY WEEK: 19990401

AB **Endostatin** is a Mr 20,000 COOH-terminal fragment of collagen XVIII that inhibits the growth of several primary tumors. We report here the cloning and expression of mouse **endostatin** in both prokaryotic and eukaryotic expression systems. Soluble recombinant protein expressed in yeast (15-20 mg/L) inhibited the proliferation and migration of endothelial cells in response to stimulation by basic fibroblast growth factor. A rabbit polyclonal antibody was raised that showed positive immunoreactivity to the recombinant protein expressed from both systems. Importantly, the biological activity of the mouse recombinant protein could be neutralized by this antiserum in both endothelial proliferation and chorioallantoic membrane assays. Systemic administration of **endostatin** at 10 mg/kg suppressed the growth of renal cell cancer in a nude mouse model. The inhibition of tumor growth with soluble yeast-produced protein was comparable to that obtained with non-refolded precipitated protein expressed from bacteria. In addition, two closely related COOH-terminal deletion **mutants** of **endostatin** were also tested and showed strikingly differing activity. Collectively, these findings demonstrate the expression of a biologically active form of mouse **endostatin** in yeast, define a role for the molecule in inhibiting endothelial cell migration, extend its antitumor effects to renal cell carcinoma, and provide a formal proof (via the neutralizing antiserum experiments and the **mutant** data) that **endostatin** (and not a possible contaminant) acts as an antiangiogenic agent. Finally, the high level expression of mouse **endostatin** in yeast serves as an endotoxin free, soluble source of protein for fundamental studies on the mechanisms of tumor growth suppression by angiogenesis inhibitors.

L3 ANSWER 12 OF 14 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 1998320342 MEDLINE
Searcher : Shears 308-4994

DOCUMENT NUMBER: 98320342
TITLE: Therapy for non-small cell lung cancer: new concepts based on molecular biology.
AUTHOR: Tanaka F; Wada H; Hitomi S
CORPORATE SOURCE: Department of Thoracic Surgery, Chest Disease Research Institute, Kyoto University, Japan.
SOURCE: NIPPON GEKA GAKKAI ZASSHI. JOURNAL OF JAPAN SURGICAL SOCIETY, (1998 May) 99 (5) 285-90. Ref: 20
Journal code: NGG. ISSN: 0301-4894.
PUB. COUNTRY: Japan
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW LITERATURE)
LANGUAGE: Japanese
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199812
ENTRY WEEK: 19981201

AB Recent advances in molecular biology have broadened our knowledge of the biological characteristics of cancer. In the present paper, we review and discuss new modalities of therapy for non-small cell lung cancer (NSCLC) based on biological findings. These modalities include: 1) diagnosis of cancer based on gene abnormalities: 2) decision making on chemo-/radiotherapy based on new biological findings: 3) gene therapy: and 4) new chemotherapeutic agents. **Mutation** of the p53 gene, which occurs most frequently in NSCLC, is a well-documented molecular target in these modalities. The development of polymerase chain reaction technology has enabled early diagnosis of NSCLC by detection of p53 gene abnormalities in sputum. Transfer of the wild-type p53 gene using a retrovirus vector to cancer tissues with **mutant** p53 gene has already been tested clinically. Inhibition of tumor neovascularization has been studied extensively in attempts to develop noveal chemotherapeutic agents. Angiostatin or **endostatin**, an inhibitor of tumor neovascularization is in clinical use. Matrix metalloprotease inhibitions (MMPs) also inhibit neovascularization of tumors. Marimastat, an oral MMP, is expected to prevent cancer metastasis.

L3 ANSWER 13 OF 14 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 1999032827 MEDLINE
DOCUMENT NUMBER: 99032827
TITLE: Zinc-binding of endostatin is essential for its antiangiogenic activity.
AUTHOR: Boehm T; O'reilly M S; Keough K; Shiloach J; Shapiro R; Folkman J
CORPORATE SOURCE: The Children's Hospital, and Departments of Surgery and Cellular Biology, Harvard Medical School, 300 Longwood Avenue, Boston, Massachusetts, 02115, USA.. boehm_t@a1.tch.harvard.edu
SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS,

(1998 Nov 9) 252 (1) 190-4.
Journal code: 9Y8. ISSN: 0006-291X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199902
ENTRY WEEK: 19990204

AB **Endostatin** is a potent angiogenesis inhibitor in vitro and in vivo. We used the yeast Pichia pastoris to express and purify soluble **endostatin**. It was discovered that metal chelating agents can induce N-terminal degradation of **endostatin**. We theorized that a metal was removed from **endostatin** which changed the conformation and allowed a contaminating protease to degrade the N-terminus. Atomic absorption and amino acid analysis of **endostatin** purified from Pichia pastoris and mammalian cells showed a 1:1 molar ratio of Zn2+ to protein. Ding et al. have shown that histidines 1, 3, 11, and aspartic acid 76 coordinate the Zn2+ atom (1). An H1/3A double, an H11A, and a D76A single **mutant** of **endostatin** were not able to regress Lewis lung carcinoma. We conclude that the ability of **endostatin** to bind Zn2+ is essential for its antiangiogenic activity. Copyright 1998 Academic Press.

L3 ANSWER 14 OF 14 MEDLINE DUPLICATE 9

ACCESSION NUMBER: 1998049348 MEDLINE
DOCUMENT NUMBER: 98049348

TITLE: Antiangiogenic therapy of experimental cancer does not induce acquired drug resistance [see comments].

COMMENT: Comment in: Nature 1997 Nov 27;390(6658):335-6
Comment in: Nature 1998 Jan 29;;391(6666):450
Comment in: Nature 1998 May 14;393(6681):97

AUTHOR: Boehm T; Folkman J; Browder T; O'Reilly M S
CORPORATE SOURCE: Department of Surgery, Harvard Medical School, Boston, Massachusetts 02115, USA..
boehmvt@a1.tch.harvard.edu

SOURCE: NATURE, (1997 Nov 27) 390 (6658) 404-7.
Journal code: NSC. ISSN: 0028-0836.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Cancer Journals; Priority Journals
ENTRY MONTH: 199802

AB Acquired drug resistance is a major problem in the treatment of cancer. Of the more than 500,000 annual deaths from cancer in the United States, many follow the development of resistance to chemotherapy. The emergence of resistance depends in part on the genetic instability, heterogeneity and high **mutational** rate of tumour cells. In contrast, endothelial cells are genetically

stable, homogeneous and have a low **mutational** rate. Therefore, antiangiogenic therapy directed against a tumour's endothelial cells should, in principle, induce little or no drug resistance. **Endostatin**, a potent angiogenesis inhibitor, was administered to mice bearing Lewis lung carcinoma, T241 fibrosarcoma or B16F10 melanoma. Treatment was stopped when tumours had regressed. Tumours were then allowed to re-grow and **endostatin** therapy was resumed. After 6, 4 or 2 treatment cycles, respectively, no tumours recurred after discontinuation of therapy. These experiments show that drug resistance does not develop in three tumour types treated with a potent angiogenesis inhibitor. An unexpected finding is that repeated cycles of antiangiogenic therapy are followed by prolonged tumour dormancy without further therapy.

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